ROYAL SOCIETY.

REPORTS TO THE MALARIA COMMITTEE.

SIXTH SERIES.

CONTENTS.

LONDON:
HARRISON AND SONS, ST. MARTIN'S LANE,
PRINTERS IN ORDINARY TO HIS MAJESTY.

1902.

Price One Shilling.

MARCH 6, 1902.

Fb 11, 87

REPORTS

TO

THE MALARIA COMMITTEE

OF

THE ROYAL SOCIETY.

SIXTH SERIES.

LONDON:
HARRISON AND SONS, ST. MARTIN'S LANE,
PRINTERS IN ORDINARY TO HIS MAJESTY.

1902.

CONTENTS.

REPORTS, &c., FROM Messrs. STEPHENS AND CHRISTOPHERS, BENGAL.

"The Relation of Malarial Endemicity to '*Species*' of Anopheles." By J. W. W. Stephens, M.D. Cantab., and S. R. Christophers, M.B. Vict. Received October, 1901.

1. *Definition of Endemicity.*

It has always been recognised that in a particular country certain districts are more malarial than others. If we proceed to ascertain to what extent malaria prevails in a district we may do so in several ways—

(1.) We may consult the hospital statistics for admissions for malaria.

(2.) We may determine to what extent enlargement of the spleen prevails.

(3.) We may determine by actual blood examination how many individuals have parasites in the peripheral circulation. More especially does the parasite rate in children, as pointed out by Koch, give a definite and true index of endemicity, which may be used in the comparison of one locality with another.

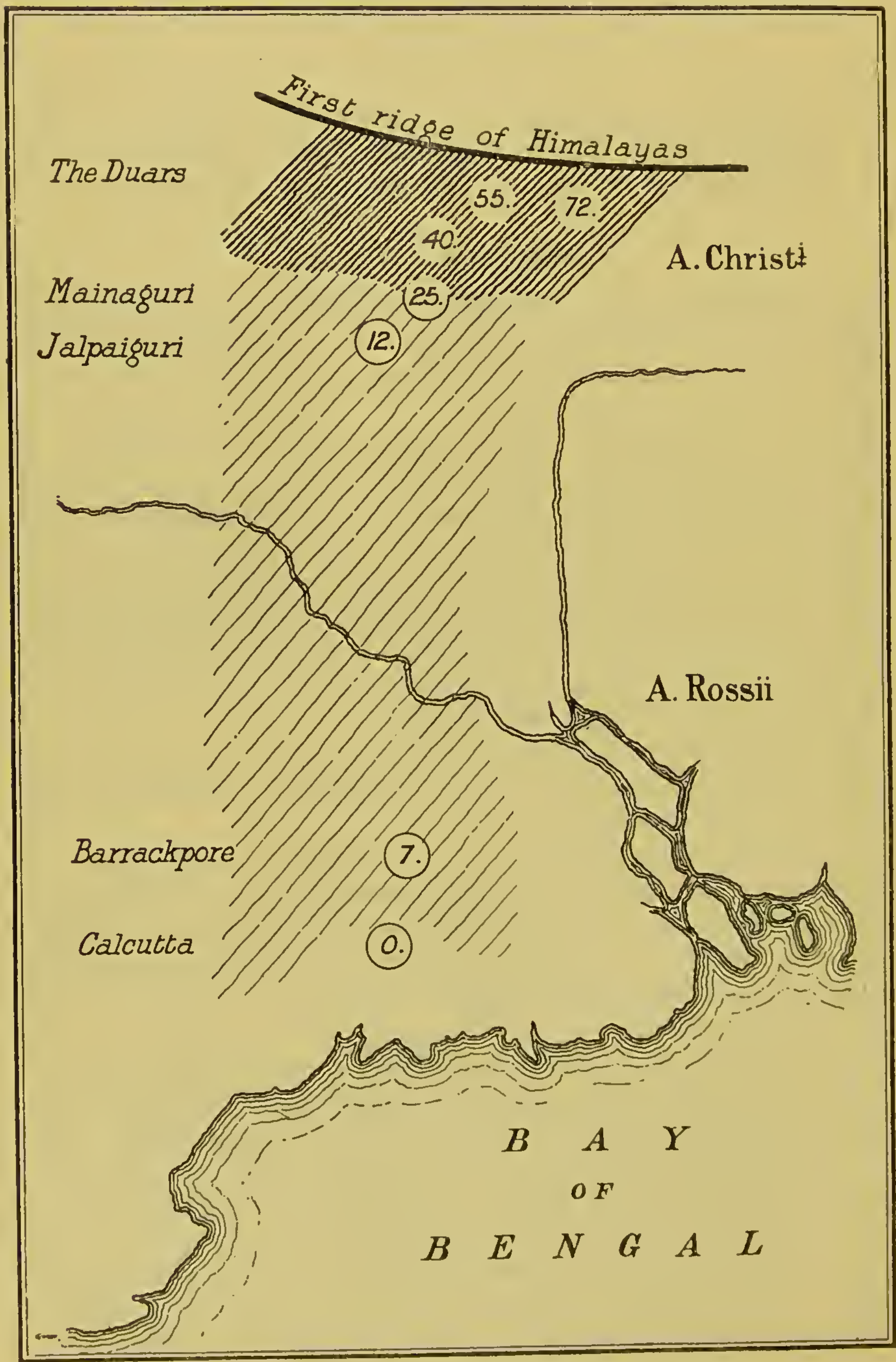
To this method we would add, as a complementary one, the determination of the percentage of infected Anopheles, as giving the actual risk of infection in a district.

These methods of determining the endemicity are by no means of equal value. The two first we shall further discuss in another paper. We may say shortly, however, that the first is of quite secondary importance and open to grave errors, whilst the second, though of value in districts where blood examinations cannot be made, yet is also open to serious error.

It is in the third sense that "endemicity" or "endemic index" is used by us in this paper. The endemic index, then, we may take to be the percentage of infected children (under 10 years of age) in any district, and is the truest test of the infection present and the liability of immigrants to contract malaria. In this way Koch has shown the endemicity of malaria to vary in the Dutch East Indies from 0 per cent. to 100 per cent. Our own researches and those of Annett and Dutton have shown that in West Africa the endemicity of the various districts is uniformly very high—20 per cent. to 100 per cent.

2. The Malarial Endemicity of Bengal.

By the examination of a number of children from various districts we have determined the endemicity of representative portions of Bengal. The following map and table will at once show how, in pro-



Sketch Map to show Variation of Endemicity in Bengal.

Table I.—Showing Variations in Endemicity in Bengal.

Locality.		Endemic index.	Number of children examined.
Calcutta	Phoolbagan Road	0 per cent.	57
	Hastings	0 "	84
	Kidderpore	0 "	50
Plains of Bengal	Barrackpore	7·7 "	26
	Belghurria	7·4 "	27
	Jalpaiguri	12·7 "	86
	Mainaguri	25 "	40
Duars	Rangamutty	43 "	72
	Nagasuri	55·5 "	27
	Ghatia	72 "	25

ceeding from Calcutta northwards till the foot of the Himalayas was reached—a distance of some 300 miles—we passed from a region the endemic index of which was 0 per cent., through regions with increasing indices of endemicity, till at the foot of the hills, in a district known as the Duars, a very high degree of infection was reached, 40 per cent. to 72 per cent., as high, indeed, as that found by us in West Africa. We have, then, a region, not above 300 miles in latitude, subject to almost identical climatic influences, with an endemic index varying from 0 per cent. to 72 per cent.

3. *The Cause of the Variation in the Endemicity of Bengal.*

As just mentioned, the climatic influences are very similar throughout the district. A high degree of atmospheric humidity, a very uniform high temperature—86° to 90° in the shade—and the presence of abundant surface water. In these respects we could see no essential difference between regions of low and those of high endemicity. We are left, then, with the following possible explanations of the variation:—

a. The number of Anopheles present.
b. The effect of the importation of non-immunised individuals.
c. The "species" of Anopheles in different districts are not the same.

a. The Number of Anopheles Present.—The portions of Calcutta investigated by us were portions of the native town, in both central and outlying districts, which, from their squalid nature, appeared the most likely to possess high fever rates. Here we had, at first sight, all the conditions which, from their similarity to those of West Africa, would have led us to expect a high endemic index. The temperature

during some months had been as high as 86° to 90° in the shade during the day, and the atmospheric humidity was as great as that of the most malarious districts of the West African Coast.

The houses were in no essential particular different from those in Africa, nor was the social condition of the inhabitants higher.

Anopheles were everywhere present, and in some quarters were extraordinarily abundant. Even in Africa we have never seen larvæ so abundant or adult insects so readily caught.

We had, then, here apparently all the essential points of an African village reproduced, and we naturally expected a considerable degree of child infection. But this proved not to be the case.

(1.) An examination of the children of several of the worst districts and those with most Anopheles was completely negative. In all, 141 children were examined throughout June, July, and August without a single parasite being found by us.

We may note that, though during this time many cases of enlarged spleen were admitted into the different civil hospitals of Calcutta and diagnosed as malaria, yet parasites were only found with the greatest rarity. The only hospitals in which parasites were readily found by us were the military hospitals.

(2.) An examination of the Anopheles from these quarters of Calcutta was also completely negative. In all, 324 Anopheles were dissected, and neither sporozoits nor zygotes were found by us.

Table II.—Showing Absence of Malarial Infection in Calcutta.

District of Calcutta.	Endemic index.	No. of children.	Sporozoit rate.	No. of anopheles.
Phoolbagan Road	0 per cent.	57	0 per cent.	200
Hastings...	,,	77	..	5
Fort William........	,,	7	0 per cent.	116
Kidderpore..........	,,	50	..	8
Total...........	..	191	..	329

Briefly, we may say that in Calcutta we had in June, July, and August an extraordinary number of Anopheles with an endemic index of 0°.

A very different state of affairs were present in the Duars. Here, though frequently we had great difficulty in collecting sufficient Anopheles to enable us to determine the sporozoit rate, the amount of malarial infection was very large. Whereas in Calcutta, in two districts at least, we were able to collect in an hour 50 or more Anopheles, in Nagasuri, in the Duars, with an endemic index of 55 per

cent., we collected during many days and with difficulty only 39 specimens.

It is evident, then, that the mere number of Anopheles present cannot be the cause of the great variation in the endemicity of Bengal.

b. The Effect of the Importation of Non-immunised Individuals.—Koch has shown how the importation of fresh coolies from the non-malarial districts is responsible for an increase in the fever incidence, and that an immunity is later developed in these, so that a gradually diminishing fever incidence ensues, until a new set of coolies is again imported.

No large immigration occurs in the district under consideration, except on the tea plantations of the Duars. As this district is the one with the highest fever rate, it is possible that the extremely high endemicity may be partly due to this cause. The endemic index taken as the test of each district was, however, determined from the coolies coming from the plains who dwelt in separate villages from the hill coolies. Moreover, the coolies from the plains were for the most part long resident in the district, and most of the children examined by us were either born in the district or had lived nearly the whole of their life there.

There was also already a marked increase in endemicity (25 per cent.) at Mainaguri, about 30 miles from the Duars. Here there is no immigration, and the inhabitants are all long resident in the district.

It seems probable, then, that over and above the effect of the immigration of strangers, there is a real and marked increase in the endemic index in this district.

c. The Species of Anopheles in Bengal.—A closer examination of the Anopheles occurring in Bengal proved that we were dealing with a considerable number of species. It also became evident that the distribution of certain species coincided with areas of high endemicity, whilst other species occurred and even existed in profusion where very little infection was present.

The species of Anopheles found by us were six in number : *A. Rossii* (Giles), *A. fuliginosus* (Giles), *A. sinensis* (Wied), sub-sp. *nigerrimus* (Giles), *A. Lindesayi* (Giles), and two species new to India. These latter are *A. metaboles* (Theobald) and *A. Christophersi* (Theobald).

It was found that in many parts of Calcutta one species only occurred from June to August, namely *A. Rossii* This species was present in enormous numbers.

In certain outlying portions of Calcutta, and in the country around Calcutta, two other species were found somewhat sparingly, namely, *A. fuliginosus* and *A. sinensis*, sub-sp. *nigerrimus*.

These three species appear to be present everywhere in the plains of Bengal. They were the only species found by us until the foot of the mountains (Duars) were reached. *A. Rossii* was also found by us at a

height of 5000 feet, *A. fuliginosus* at 800 feet, and *A. nigerrimus* at 1200 feet North of Jalpaiguri, about 30 miles from Duars, *A. metaboles* was found breeding scantily. This species was found to increase in numbers as we passed northwards, and it extended high into the hills (5000 feet).

The district known as the Duars is a strip of land extending for some hundreds of miles along the foot of the Himalayas. It is a gently sloping tract of from 800 feet to 1200 feet above the sea, and abounding in small streams. In this narrow strip another new species, *A. Christophersi*, was found in abundance by us. This is a very small mosquito and resembles to some extent *A. funestus*. We have never found this species in any of the districts visited by us, except in the Duars.

A. metaboles was in the Duars also a very common mosquito, judging by the large numbers and wide distribution of larvæ.

Our observations so far then can be stated briefly as follows :—

Table III.—To show Relation of Species of Anopheles to Endemicity.

Locality.	Species present.	Endemic index.
Calcutta { Phoolbagan Road....	*A. Rossii.*	0 per cent.
Calcutta { Hastings	*A. Rossii.* *A. fuliginosus.*	0 per cent.
Plains of Bengal..............	*A. Rossii.* *A. fuliginosus.* *A. nigerrimus.*	From 7 to 12 per cent.
Duars	*A. Christophersi.* *A. metaboles.* *A. Rossii.*	From 40 to 72 per cent.
Hills (5000 feet)..............	*A. metaboles.* *A. Rossii.* *A. Lindesayi.*	0 per cent.

Such facts led us to question whether considerable variation did not exist in the power of different species of anopheles to act as host to the malarial parasites, and whether the endemicity in any district might not, to a large degree, be dependent on which species of anopheles occurred there.

We therefore wished to determine for each of the species whether, in the first place, it was a carrier of malaria. Unfortunately, in the Duars we had few facilities for feeding, and the evidence for some of the species is not conclusive. The following experiments and observations show, however, that *A. Rossii* appears to be not a carrier, or in any case a very poor carrier, that *A. Christophersi* is a good carrier, and that *A. fuliginosus* and *A. nigerrimus* are doubtful as carriers.

A. Rossii.—The fact that this species can occur in such profusion as it did in Calcutta without a corresponding degree of infection, is in itself strongly suggestive that this species is a harmless one.

We may note also that Major Ross was unable to cultivate human malaria in Calcutta, where he must have used *A. Rossii*, but that he succeeded in doing so with some specimens of Anopheles up country.

Our own experiments with *A. Rossii* have not been more successful. The insects used by us were not reared from larvæ, but caught already fertilised in sheds and houses. Any possible infection in these would in no way vitiate the experiment, and by this means we avoided the risk of non-fertilisation or other influences which render the use of bred insects uncertain.

The following table gives the result of our experiments with *A. Rossii*:—

Table IV.—To show Result of Feeding *A. Rossii* upon Cases of Malaria.

Case.	Date of feeding.	Date examined.	Result.	Number examined.
Simple tertian ...... Numerous large forms.	July 20, 21, 22, 23	July 25	Negative	8
Crescents .... Very abundant throughout.	July 21, 22, 24	July 26	Negative	5
Simple tertian ...... Numerous large forms.	July 22, 23........	July 25	Negative	6
Simple tertian .... Fairly numerous large forms.	July 24, 26........	July 27	Negative	9

As *A. fuliginosus* and *A. nigerrimus* seemed to be the only alternative carriers of malaria in the regions of low endemicity around Calcutta, as many specimens as possible were examined. In an accompanying paper we have shown that though *A. nigerrimus* is a very common species breeding extensively over large tracts of marsh, yet that in houses it was seldom caught. The difficulty of obtaining specimens prevented our determining whether this species carried sporozoits. A small number (four) caught in houses were fed every night for five nights on a case which had crescents in large numbers. These were examined on the fifth day, but were negative. Eight specimens caught in houses were also negative.

A. fuliginosus were more abundant in houses, but still rather rare. Thirty-six specimens of *A. fuliginosus* were found negative. Two specimens were dissected on the fifth day, after feeding on a crescent case, but were negative.

A. Christophersi.—This species is undoubtedly a good carrier. Sporozoits were found in 4 out of 64 specimens, or 6·25 per cent. This rate is only a little lower than that frequently found by us in Africa.

A. metaboles.—From the difficulty of obtaining the adult insects, sufficient numbers were not examined to determine whether this species carried malaria. Eleven specimens from huts were negative.

It was hoped that sufficient specimens of the five species might have been examined in an area of high endemicity to give figures which would show the sporozoit rate of each species. We were, however, at the present time (August) unable to do so. We believed, however, that marked differences would be found, and we believed this to be the explanation of certain observations made by us in Africa. Thus Lagos had a sporozoit rate of only 3 per cent., while 50 miles up country a sporozoit rate of 50 per cent. occurred. In Lagos we were dealing almost exclusively with *A. costalis*, while in the latter place the common species was *A. funestus.*

Before drawing the conclusion that the endemicity of a district is dependent on the species of Anopheles present, much more extended observations than those we have now put forward are required. Even a district like Bengal is in reality so extensive that generalisations are apt to be hasty. We can, however, draw the following conclusions, and they show that the question of "species" is a very important one, and one that may possibly be an important cause in bringing about such varying degrees of endemicity as are found in different districts.

Conclusions.

1. That *A. Rossii* occurs in Calcutta in June, July, and August, together with an endemic index of 0 per cent. and a sporozoit rate of 0 per cent. That it is difficult to cultivate malaria experimentally in this species.

2. That the distribution of *A. Christophersi* corresponds closely with an area of extremely high endemicity. That the sporozoit rate in this species is as high as is usually found in tropical Africa.

We may point out also that the *species of parasite* should also be taken into consideration. It is manifestly not accurate to compare these with one another as simply so many cases of malaria. It is possible that all three parasites do not develop with equal facility in the different species of Anopheles. The question of "*species*" of Anopheles in relation to malaria requires to be worked out much more carefully than has yet been done.

"Some Points in the Biology of the Species of Anopheles found in Bengal." By J. W. W. STEPHENS, M.D. Cantab., and S. R. CHRISTOPHERS, M.B. Vict. Received October, 1901. (The Determination of the Species by F. V. THEOBALD, M.A.)

In an accompanying Report (The Relation of Endemicity of Malaria to the Species of Anopheles) we show that the power of transmitting malaria appears not to be possessed in the same degree by all species, and whilst it is even probable that some species may not be concerned at all in the spread of malaria, the presence of other species is associated with a high rate of malarial endemicity.

It is very necessary, then, that the habits of life of each species of Anopheles should be determined, as pointed out by Nuttall, not in general terms but for each particular species.

In the present paper we note such differences as were observed by us in the habits of the species of Anopheles which were found by us in Bengal. These were six in number—*Anopheles Rossii* (Giles), *Anopheles fuliginosus* (Giles), *Anopheles sinensis*, sub-sp. *nigerrimus* (Giles), *Anopheles Lindesayi* (Giles), and two new species. These latter resemble *Anopheles costalis* and *Anopheles funestus*, but specimens sent to Theobald were found by him not to be these species. They are described by Theobald as *Anopheles metaboles* and *A. Christophersi.* It was found possible to distinguish both the eggs and larvæ of these species. The eggs of the species under discussion vary more than the larvæ, and, indeed, could sometimes be distinguished by the unaided eye. The eggs of two species—*A. nigerrimus** and *A. Christophersi*—are characterised by an extremely narrow upper surface (see fig. 3, *c*, *d*). Those of *A. Rossii* are unique in possessing a broad fringe-like structure arising from the borders of the upper surface (see fig. 3, *a*).

The larvæ may be distinguished by an examination of the antennæ, of four small hairs hanging over the mouth, of the number of segments bearing palmate hairs, and of the individual leaflets of the palmate hairs. The presence of a large branched hair arising from the inner border of the antenna occurs in only one of the species—*A. nigerrimus.* The four small hairs over the mouth have been noted by Grassi as presenting differences of specific importance.

In the species under discussion the central pair do not present any marked differences. The outer pair, however, may be simple as in *A. Rossii*, *A. metaboles*, or they may be branched as in *A. nigerrimus*, *A. fuliginosus*, and *A. Christophersi*, so as to form a cocade-like tuft over the feeding brushes.

* *A. nigerrimus* is used for brevity; it should read all through as *A. sinensis*, sub-sp. *nigerrimus*. (F. V. T.)

Fully developed palmate hairs are as a rule confined to the last five abdominal segments. A small imperfectly formed hair is also frequently present on the 2nd abdominal segment. In *A. Christophersi*, however, every abdominal segment bears a fully formed palmate hair, and there is an imperfect pair situated on the posterior portion of the thorax. *A. Lindesayi* also has fully developed palmate hairs on the last six abdominal segments.

A palmate hair consists of from 14 to 20 leaflets, arranged so as to form a structure resembling the leaf of a Palmyra palm. The leaflets, towards their distal extremities, are more or less jagged or notched, and, except in *A. nigerrimus*, are continued past these notches only as a filament considerably narrower than the basal portion of the leaflet. The character of the notching and the length of the terminal filament compared with the basal portion were found by us to be of specific nature (see fig. 1).

The following is a description of the main differences in the eggs and larvæ of the species under discussion :—

A. Rossii (see figs. 1, 2, and 3).

Egg.—Anterior surface very broad.

Frill very wide and continued around the edge of the upper surface so that it passes above the floats.

Floats are of the shape of a scallop-shell and reach the border of the upper surface.

Lower surface of egg without distinct polygonal markings.

Larva.—Antenna without large lateral branched hair.

Hairs over mouth unbranched.

Palmate hairs borne by 3rd, 4th, 5th, 6th, and 7th abdominal segments. Imperfectly formed palmate hair on 2nd abdominal segment.

Palmate hairs rather small. The terminal filament half the length of the whole leaflet.

A. fuliginosus (see fig. 3, *b*).

Egg.—Upper surface rather narrow.

Fringe rudimentary. Fringe is not continued above floats, being wanting in the portion of the edge of the upper surface where the floats are placed.

Floats regularly oval and touch margin of upper surface.

Lower surface of egg not marked by definite polygonal areas.

Larva.—Antenna without large lateral branched hair.

Outer pair of hairs over mouth developed into a tuft.

Palmate hairs borne by 3rd, 4th, 5th, 6th, and 7th segments.

Palmate hairs dark in colour, very markedly jagged. Terminal filament not so long as basal portion.

A. sinensis, sub-sp. *nigerrimus*. (See figs. 1, 2, and 3.)

Egg.—Upper surface very narrow.

Fringe rudimentary. Passes all round margin of upper surface.

Floats oval.

Floats do not reach margin of upper surface, but leave a portion of lower surface of egg intervening.

Lower surface marked by silvery lines into polygonal areas.

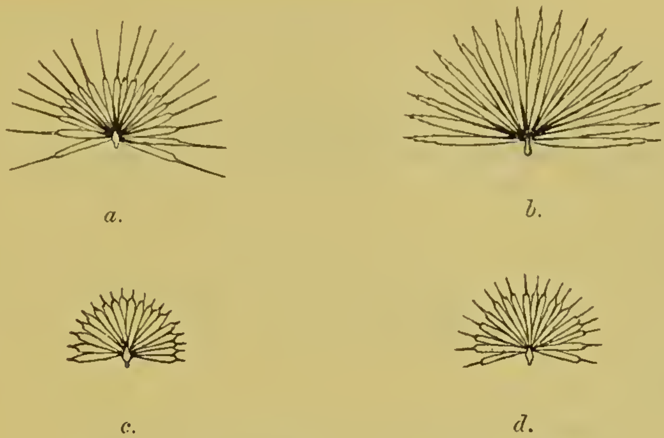


Fig. 1.—Palmate Hairs of Larval Anopheles.

a. A. Rossii ; b. A. nigerrimus ; c. A. metaboles ; d. A. Christophersi.

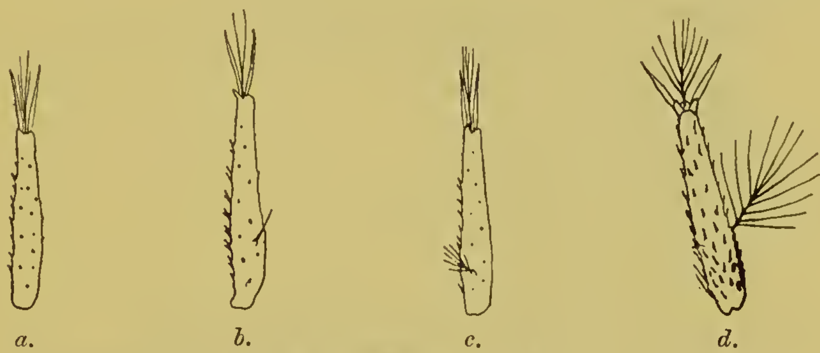


Fig. 2.—Antennæ of Larvæ.

a. A. Rossii ; b. A. metaboles ; c. A. Lindesayi ; d. A. nigerrimus.

Fig. 3.—Ova of Anopheles.

a. A. Rossii ; b. A. fuliginosus ; c. A. nigerrimus ; d. A. Christophersi.

Larva.—Antenna with large branched hair arising from inner border.

Outer pair of hairs over mouth developed into large very prominent cocade-like tufts.

Palmate hairs borne by 3rd, 4th, 5th, and 6th abdominal segments. Imperfect palmate hair on 2nd segment.

Palmate hairs very large. Leaflets lanceolate in shape, with the terminal third deeply serrated.

A. Lindesayi.

Egg.—Not observed.

Larva.—Antenna with small rudimentary branched hair. (Fig. 2, *c.*)

Hairs over mouth unbranched.

Palmate hairs borne by 2nd. 3rd, 4th, 5th, 6th, and 7th abdominal segments.

Palmate hairs large.

Terminal filament long, but not so long as basal portion.

A. metaboles (see figs. 1 and 2, *c* and *b*).

Egg.—Not observed.

Larva.—Antenna with rudimentary hair, unbranched.

Hairs over mouth unbranched.

Palmate hairs borne by 3rd, 4th, 5th, 6th, and 7th abdominal segments. Imperfect hair on 2nd segment.

Palmate hairs rather large.

Terminal filament very short.

A. Christophersi (see figs. 1 and 3, *d*, *d*).

Egg.—Egg smaller than in other species.

Upper surface very narrow.

Fringe rudimentary.

Floats do not touch edge of upper surface.

No polygonal markings on the lower surface of the egg.

Larva.—Antenna without large lateral branched hair.

Outer pair of hairs over mouth developed into small tuft.

Palmate hairs borne by all abdominal segments. Imperfect palmate hair on thorax.

Palmate hairs large. Terminal filament rather short, but longer than that of *A. metaboles.*

The distribution of the Species.—*A. Rossii* was found everywhere by us—from Calcutta to the foot of the Himalayas, and at an elevation of 3000 feet. In the Duars it was comparatively rare. In Calcutta, on the contrary, it was by far the commonest species, and in many parts of Calcutta appeared to be the only species present.

A. Christophersi was only found by us in the Duars district, and it was not found above a height of about 1000 feet.

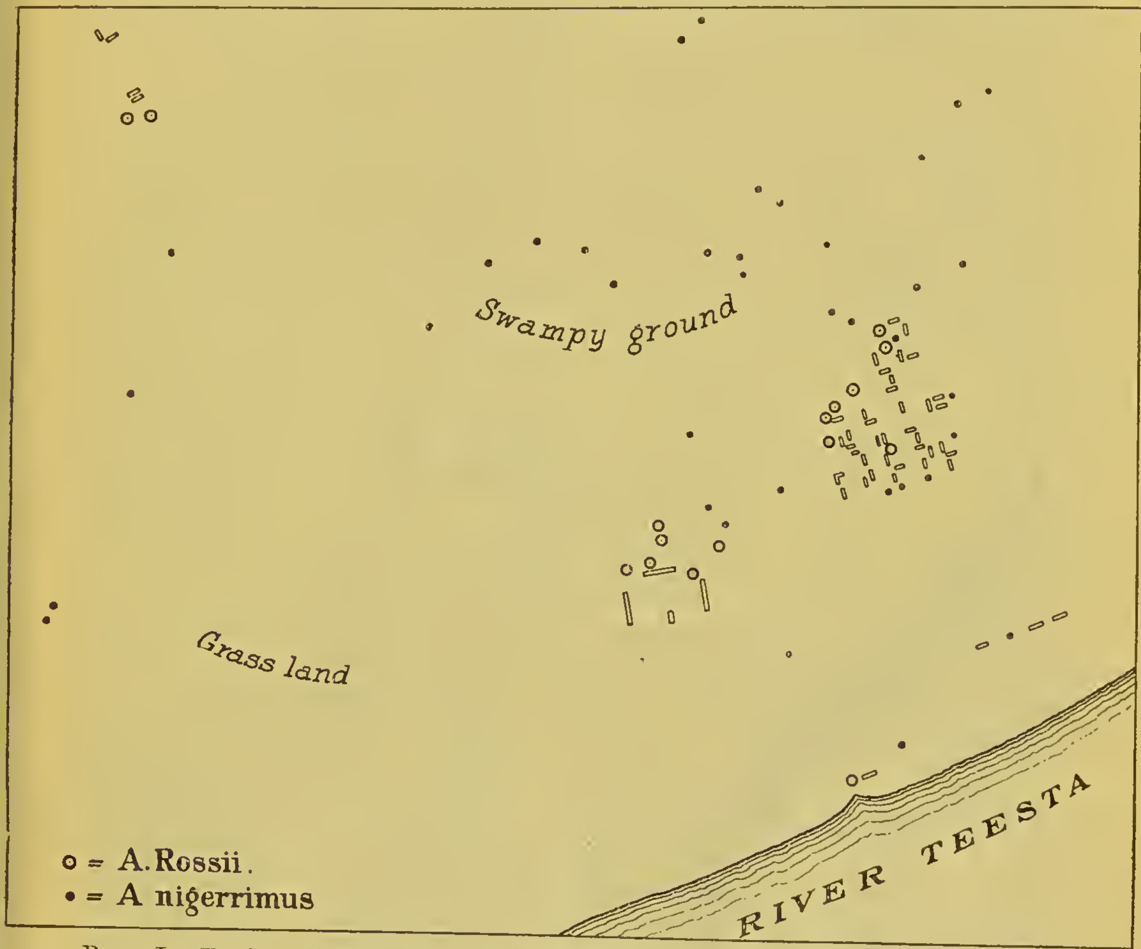
A. metaboles occurred in small numbers as one approached the foot of the hills. It was the commonest Anopheles in the upper portions of the Duars and lower hills. It occurred also at an elevation of nearly 5000 feet near Kurseong.

A. fuliginosus was found scantily in the plains and at a height of 800 feet.

A. nigerrimus was, except in Calcutta, a common species in the plains, and larvæ were found at a height of 1200 feet in the Duars.

Habits of the different Species.

A. Rossii.—*A. Rossii* was found by us always near human dwellings, and often in very foul water. In spite of the commonness of the species, larvæ were never found by us more than a stone's-throw from dwellings. If in any place larvæ were discovered at a greater distance



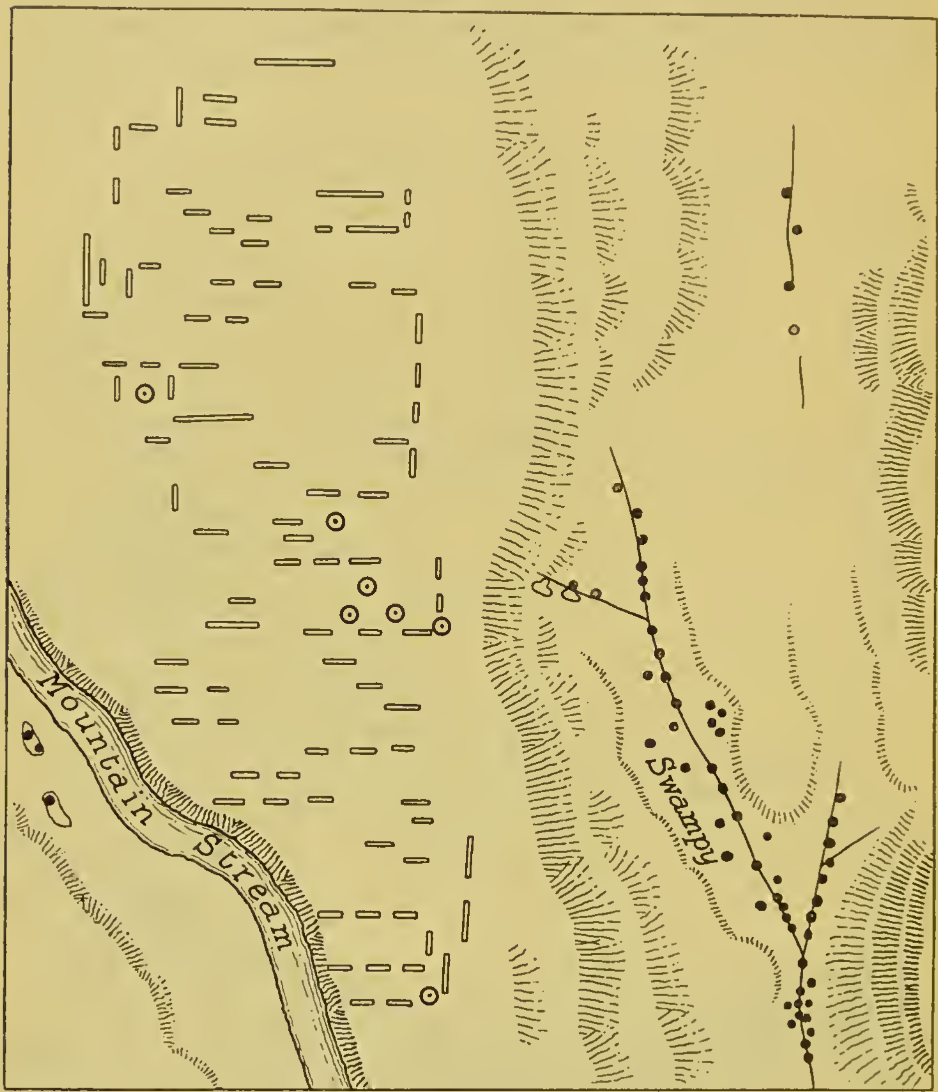
Plan I.—To show distribution of breeding-places of *A. Rossii* and of *A. nigerrimus.* Open circles = *A. Rossii*; solid circles = *A. nigerrimus.*

they invariably turned out to be larvæ of other species. *A. Rossii* appears, then, in Bengal to be "foveal" in its distribution, in contra-distinction to other species of Anopheles to be described.

In Plan I the distribution of *A. Rossii* breeding-places can be compared with those of *A. nigerrimus,* in Plan II with those of *A. metaboles* and *A. Christophersi.*

In captivity *A. Rossii* lays eggs more readily than *A. fuliginosus* or *A. nigerrimus.* The eggs laid on mud were sometimes deposited in a heaped up mass, in others scattered over the surface of the mud. Some of the eggs of *A. Rossii* do not become black, and these appear

to dry up quickly and do not develop. Sometimes the whole of the eggs laid are of this nature, at others a certain number are normal, whilst others remain white or change only to a reddish tinge.



⊙ = *A. Rossii.*
● = *A. Christophersi* and *A. metaboles.*

PLAN II.—Portion of Coolie Lines on a Tea Plantation—to show breeding-places of *A. Rossii*, *A. Christophersi*, and *A. metaboles.*
Open circles = *A Rossii*; solid circles = *A. Christophersi* and *A. metaboles.*

The eggs of *A. Rossii* possess very slight powers of resisting desiccation. Gravid females of *A. Rossii* were allowed to deposit eggs upon moist mud in a large earthenware vessel. The mud was then allowed to dry out of doors. From time to time portions of mud were removed and the eggs floated on to the surface of water in such

a way as not to wet them. At the end of 24 hours the surface of the mud was almost dry. Eggs removed and placed in water hatched in a few minutes. At the end of 48 hours the mud was quite dry. Eggs removed and added to water hatched in a few minutes. On the third day eggs removed and added to water did not hatch. On the fourth day the whole mass, with many hundreds of eggs upon its surface, was carefully moistened, but no larvæ hatched out. There does not appear to be, then, any probability of the eggs of *A. Rossii* resisting even a short period of drought in the course of nature.

The larvæ of *A. Rossii* usually occur in very large numbers in the pools they frequent. Other species do not seem to breed in such a crowded state. The food of the larvæ varied somewhat in different situations. In all there was much sand. In larvæ from shallow newly formed rain pools on mud, the gut contents were largely composed of bacilli and small infusoria. In water of a more permanent nature, algæ of several species, and a great variety of unicellular green vegetable cells, were found. Diatoms, though present, rarely formed a large proportion of the food.

The larvæ of *A. Rossii* appear unable to resist drying, even for a few hours. A number allowed to remain on drying mud very quickly died.

Experiments were made to determine how far *A. Rossii* selected a particular kind of water for the deposition of ova. A number of gravid females were placed in a large mosquito net along with earthenware vessels containing different waters. It was found that very little if any choice was shown between pure water, green stagnant water, or even very foul sewage, eggs having been laid in the same night in each of these vessels.

A. Rossii will readily lay eggs on half dry or dry mud, and confined in a dry tube or bottle will lay eggs upon glass.

A few observations were also made on the natural enemies of Anopheles larvæ. While some fish (*e.g.*, a small fish which was identified by Major Alcock, I.M.S., F.R.S., as a young carp, and which occurred abundantly in the larger tanks in Calcutta) feed voraciously on larvæ, others do not eat any.

The following table will give a rough idea of the rate at which the commoner water creatures devour larvæ :—

	Number of larvæ added.	Number after 1 hour.	Number after 12 hours.
Large libellula larva	20	7	1
Small ,, ,, ...	12	7	0
Beetle (Dytiscidæ)	12	9	2
*Beetle (Clavicorn)	9	9	8
Small dytiscid larva	8	4	2
Coryxa	9	3	0
Nepa......................	16	5	2
*Tadpole (small species)......	9	9	8

From 50–100 larvæ were added to large earthenware vessels containing one or two specimens of the following, which are the commonest tank fish :—

Fish.	Observation.
2 Catfish	Larvæ gradually disappeared during several days.
6 Young barbus	Larvæ all disappeared in 15 minutes.
8 Trichogastra........	Larvæ slowly diminished.
4 Polyoeanthus	Very few larvæ eaten.
2 Small carp (adult) ..	Larvæ apparently undiminished at end of 48 hours.
A small acclimatised salt-water fish	Larvæ not eaten.

Twelve specimens of the young barbus were then added to a small cement tank containing numerous larvæ. At the end of 24 hours no larvæ were visible, and the pool remained free for 6 days, the period during which it was under observation.

It is evident that these small fish devoured larvæ far more rapidly than the other fish used, and than any of the insect enemies. This is a common fish, and is easily caught. It is, moreover, a fish not readily killed by foul water or transportation. For tanks and small collections of water, which it is undesirable to do away with, the introduction of a number of these fish would appear to be at least worth a trial. With regard to experiments with *A. Rossii* we can say :—

1. Little or no selection of water is exercised by the female in laying her eggs.

2. The eggs do not stand desiccation for more than 48 hours.

3. The larvæ are still more susceptible to desiccation.

4. Of small water animals, larvæ of libellula, beetles of carnivorous habits and their larvæ, coryxa and nepa all feed upon larvæ.

* The disappearance of a single larva after so many hours was probably accidental, as larvæ of various sizes were used.

5. Some species of fish are much more voracious feeders on larvæ than others.

A. nigerrimus.—While *A. Rossii* is foveal, *A. nigerrimus*, on the other hand, is non-foveal. Though the insects were seldom caught by us in houses, yet it is a common species, and the larvæ, unlike those of *A. Rossii*, are to be found plentifully in sluggish streams, river banks, marshes, and swampy pools.

This species was at first thought by us to be a somewhat rare species, as it seldom occurred in collections of Anopheles made in different quarters. On examining natural waters, however, we found the larvæ to be very prevalent.

Unlike the larvæ of *A. Rossii*, the larvæ of this species occur singly, and can only be detected by frequent " dipping " amidst the grass and water plants. Taking into consideration the great extent of waters that *A. nigerrimus* larvæ were found in, this species is, in the marshy parts of Bengal, one of the commonest.

The larvæ of *A. nigerrimus* were found apart from collections of huts, and though they occurred in deep water, even in the midst of villages, yet they were not more numerous in these situations than elsewhere. It is noteworthy that in one situation, where deep ditches with clean stagnant water were situated side by side with shallow foul puddles, the larvæ of *A. Rossii* were never found in the former but were numerous in the latter, whereas those of *A. nigerrimus* occurred only in the former. Although the adults of this species were only rarely found in houses, yet it readily fed upon human blood. The habits of this species therefore require elucidation.

A. Christophersi.—In the Duars this species breeds in sluggish streams with grassy edges. It was never found by us in puddles in the coolie lines, or in small pools on roads, paths, &c. In some cases larvæ were dipped up from water running with considerable velocity, but always amongst the grass at the edge. The adults were found in the coolie huts, but were difficult of detection from their small size. It is possible that this species has a more powerful flight than *A. Rossii*, as the larvæ usually occurred some distance from the huts. In one case the nearest breeding place was 200 yards away.

A. metaboles.—The larvæ of this species also occur in streams more or less rapid. They are also abundant in swampy tracts by the side of streams, in rice fields, and small pools. They are not found in the small foul pools in the coolie lines. They occur at a height of 5000 feet. This species has been found by us at very considerable distances from habitations.

A. fuliginosus.—In Fort William, Calcutta, the insects were common. Elsewhere we have very seldom caught the adults. The larvæ were found in the same situations as those of *A. nigerrimus*, but they were not so numerous.

A. Lindesayi.—This species was only found by us in the hills at a height of about 4000 feet. It was breeding together with *A. metaboles.*

Summary.

1. *A. Rossii* is a foveal species and will breed in foul water.

2. *A. nigerrimus* occurs quite apart from habitations. It only breeds in marsh water.

3. *A. metaboles* occurs from the base of the hills to 5000 feet. It breeds in pools, swampy edges of streams, and rice fields. It has been found by us at great distances from dwellings.

4. *A. Christophersi* is, in the Duars, almost confined to small streams. It does not extend very high into the hills, and in the part of Bengal seen by us is present only along the base of the hills.

"The Relation between Enlarged Spleen and Parasite Infection." By J. W. W. STEPHENS, M.D. Cantab., and S. R. CHRISTOPHERS, M.B. Vict. Received October, 1901.

Discussions have recently arisen in scientific literature on the value of the so-called "spleen test" for malaria. Arguments based on this test appear to us not conclusive; because, so far as we know, no data have hitherto appeared in which the spleen rate and parasite rate have been simultaneously determined. While engaged, then, in determining the parasite rate or endemic index of various localities in Bengal, we took the opportunity of determining the spleen rate among the children examined. The data thus simultaneously acquired, and the conclusions to which they lead us, form the subject of this Report.

It is well known that intense malaria may exist without any corresponding affection of the spleen. Thus, F. Plehn,* speaking of enlarged spleen, says: "Sie ist in der überwiegenden Mehrzahl der Fälle nicht grösser als in den von mir in Deutschland beobachteten Fällen von Abdominaltyphus, häufig wesentlich kleiner"; and Daniels† "concludes that as a test for the prevalence of malaria the spleen test may be worse than useless, unless race and age are taken into account." Ross, we believe, found in Freetown, Sierra Leone, very few enlarged spleens amongst the troops suffering from malaria there.

But in India there exists little doubt that amongst natives and Europeans enlarged spleen is one of the commonest occurrences in those suffering, or who have suffered, from malaria. For in the wards

* 'Die Kamerun-Küste,' p. 97.

† 'Thompson-Yates Reports,' 1901.

of the large native hospitals in Calcutta, among the large number of "fever" cases (in July), the great majority were classified as suffering from "malarial cachexia and enlarged spleen." These cases were briefly characterised by anæmia, with an enlargement of the spleen varying from 2—3 fingers' breadth to that of a spleen filling the whole of the left side of the abdomen, and reaching to the pubes; and these cases were quite common. These cases were also not infrequently accompanied by a temperature which oscillated in an irregular way between 99° and 101°, and which might persist for weeks. Quinine or cinchona, as far as we could see, frequently exerted no influence on the course of the temperature.

When we came to examine these cases microscopically, they consistently showed a complete absence of parasites. We examined over 80 of such cases, and in none did we find parasites, or pigmented leucocytes, or any mononuclear leucocyte increase such as we have shown in earlier Reports to be characteristic of a recent infection.

Many of these patients were taking cinchona, but others were not; and in neither were these subsidiary signs of malarial infection—pigment and a mononuclear increase—present.

Further, we had the opportunity of making *post-mortem* examinations on six cases, and in none of these were parasites found in the bone marrow or spleen; though pigment was present in variable amount.

We do not consider these to be new facts, for similar ones have been recorded by Kelsch and Kiener, Ross and Daniels, under the designation "symptomatic fever." We here wish chiefly to point to the importance of these cases from the point of view of classification. It can be of little value to classify cases of this kind (so-called malarial cachexia) with those of, say, simple tertian with numerous parasites in the circulation, under the general heading of malaria (ague or remittent fever). This classification, however, prevails in India; and for this, among other reasons, hospital and dispensary statistics are of little if any value. It can only be by a microscopic differentiation of cases according to the variety of parasite that a true classification can be founded.

In Calcutta, then, we had a series of 80 cases of enlarged spleen without any corresponding parasite infection. No doubt they represented past cases of actual infection with parasites; but, as we shall soon see, they tell us little, though swelling the hospital statistics, of the actual malarial endemicity of Calcutta. In an accompanying Report we have taken the test of the endemicity of malaria, as first shown by Koch, to be the percentage of children (under 10 years of age) infected with parasites. With the endemic index thus obtained we compare, in the following table, the spleen rate :—

Table I.—To show relation between Spleen Rate and Parasite Rate.

Locality.	Spleen rate.	Endemic index.	No. of children examined.
Calcutta	0	0	191
Jalpaiguri—			
Bustee children......	27	16·1	37 and 62
School..............	14·7	0	210 and 80
Babu children	14·2	0	21 and 21
Mainaguri	74	25	51 and 41
Rungamutty...........	83	43·6	72
Sam Sing (2000 ft.)	7·1	16	14
Kurseong I (5000 ft.) ..	0	0	25
Kurseong II (2000 ft.)..	·2	0	20

We see that there is, with some exceptions to be noted, a fair correspondence between enlarged spleen and endemic index from a spleen rate of 0 at Calcutta, with an endemic index of 0, to a spleen rate of 83 at Rungamutty, with an endemic index of 43·6. Further, we see that, with one exception, the spleen rate is higher than the endemic index. The reason of this is, no doubt, that those children who at one age-period have suffered from malaria and enlarged spleen, still have the enlarged spleen at a later age-period, when the actual parasite rate is less.

So in adults we frequently have a number of enlarged spleens without any corresponding parasite infection, as in the case of 19 enlarged spleen cases in Jalpaiguri Jail, none of which showed parasites.

The spleen rate taken without modification is not a true test of the presence of malaria. Moreover, in making comparisons it is necessary that rates for corresponding age-periods should be compared.

An analysis of Table I gives the following results :—

Table II.

	Age.	Spleen rate.	Endemic index.	Number of children.
Jalpaiguri— Bustee children..	1— 5	12·5	0	8 and 17
	6—10	10·5	16·6	19 and 24
	11—15	62·5	18·7	8 and 16
Mainaguri	1— 5	52·3	37·5	21 and 16
	6—10	85	26·6	20 and 15
	11—15	100	0	10 and 10
Rungamutty	1— 5	77·4	48·3	31
	6—10	81·5	39·4	38

With one exception, then, we see that with increasing age the endemic index decreases (this is constantly the rule as found in West Africa), yet the spleen rate goes on increasing.

A factor beyond those already mentioned may cause a variable spleen rate, namely, the kind of parasite. In India we have found the quartan most commonly in the native children examined by us. It is also generally agreed that the simple tertian causes greater enlargement of the spleen than the malignant tertian.

The following conclusions may, we think, be drawn from these facts :—

1. A high endemic index may exist without any appreciable spleen rate (Africa).
2. A high spleen rate may exist in adults without a corresponding parasite infection.
3. In India (Bengal) among children a high spleen rate is a fair indication of the parasite infection.
4. The spleen rate, unlike the parasite rate, increases up to a certain age limit, and may be considerable when the parasite rate is nil.